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Evidence that the dominant follicle of the first wave is more active than that of the second wave in terms of its growth rate, blood flow supply and steroidogenic capacity in cows

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ABSTRACT

To clarify the different characteristics of the dominant follicle (DF), the DF in first ovarian follicular wave (DF-1) after spontaneous ovulation and DF in second follicular wave (DF-2) and after induced ovulation of the first-wave DF by GnRH were examined in non-lactating Holstein cows. Follicular maturation of DF-1 and DF-2 were induced by PGF2α and GnRH treatment on Day 6 and 8 (Day 0 = Day of follicular wave emergence), respectively. Follicular growth and blood flow (BF) in the follicular wall of DF-1 and DF-2 were examined. To analyze sex steroids in follicular fluid (FF) and amount of mRNA in granulosa cells, DF-1 and DF-2 were aspirated on Day 8 or 9 in different estrous cycle. Diameter in DF-1 was larger than DF-2 on Day 8 and 9. From Day 8 to 9, BF area (BFA) and percentage of the follicular wall with BF, which represents the degree of distribution of BF, increased in DF-1 but not in DF-2. BFA per length of follicle circumference with BF, which represents the thickness of BF, was not different between DF-1 and DF-2. Concentrations of 17β-estradiol (E2) in plasma, E2 and androstendione in FF and amounts of LH receptor mRNA were greater in the DF-1 on Day 8. Gene expression for steroidogenesis, prostaglandin synthesis and angiogenesis did not differ between DF-1 and DF-2. These results indicated that DF-1 were more active than DF-2 in growth, BF supply and steroidogenesis. The greater BFA observed in the DF-1 may be derived from as a result of the greater vascularity in the follicular wall.

1. Introduction

Two or three follicular waves occur in the cow during the estrous cycle (Sirois and Fortune, 1988). The first follicular wave emerges soon after estrus and the first-wave dominant follicle (DF) is a non-ovulatory follicle because it develops during the first half of the estrous cycle simultaneously with the corpus luteum (CL; Ginther et al., 1989). In the estrous cycle with two-waves of follicular development, the second wave emerges on Days 9–10 of the estrous cycle, and the second-wave DF becomes an ovulatory follicle due to CL regression. The first-wave DF develops during a period when there are basal and increasing plasma concentrations of progesterone (P4). In contrast, the second-wave DF develops during a period when there is greater plasma concentration of P4. Thus, the first- and second-wave DF develop when there are different endocrine environments (Wolfenson et al., 1999).

Wolfenson et al. (1999) reported that the concentration of 17β-estradiol (E2) in follicular fluid (FF) and that the production of androstenedione (A4) and P4 by cultured theca

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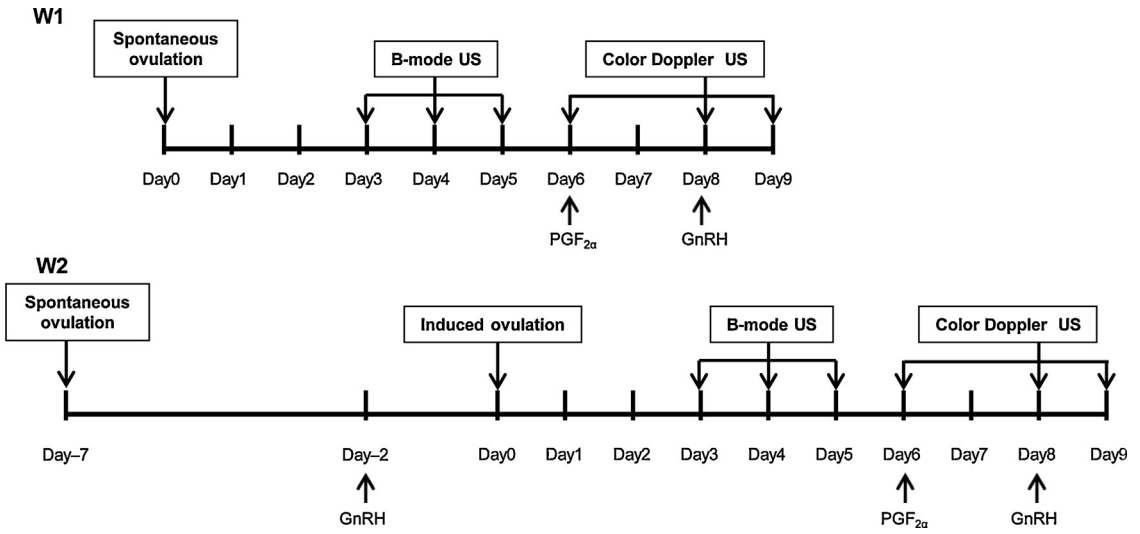

Fig. 1. Schematic diagram of experimental model of W1 and W2. In W1, the day of spontaneous ovulation was defined as Day 0. The mean diameter of DF-1 was measured on Day 3, 4, and 5. To induce follicular maturation, PGF2α and GnRH were administrated on Day 6 and 8, respectively. The mean diameter and BF of DF-1 were measured on Day 6, 8, and 9. In W2, the day of spontaneous ovulation was defined as Day-7. Ovulation from the first-wave DF was induced by GnRH treatment on Day-2 to induce a new follicular wave, and induced ovulation was confirmed 36 h after GnRH treatment on Day 0. The mean diameter of DF-2 was measured on Day 3, 4, and 5, and then PGF2α and GnRH were administrated on Day 6 and 8, respectively. The mean diameter and BF of DF-2 were also measured on Day 6, 8 and 9. Blood samples for hormone assays were collected daily from Day 0 to Day 9 from tail vein. B-mode US = trans-rectal ultrasonography. Color Doppler US = trans-rectal color Doppler ultrasonography.

cells are greater in the first-wave DF compared with those in the second-wave DF. In addition, blood flow (BF) in the wall of the pre-ovulatory follicle was greater during the first wave than the second wave (Jordan et al., 2009). The extent of BF in the pre-ovulatory follicle, which is an index for the DF quality (Siddiqui et al., 2009a,b), is greater in the first wave than that in the second wave (Jordan et al., 2009). Therefore, it is hypothesized that the quality of DF is greater in the first- than the second-wave.

However, the exact duration of follicular growth of the first- and second-wave DF after follicular wave emergence was unclear in these reports. The differences in growth, BF supply, and steroidogenic capacity were not appropriately evaluated with regard to time-dependent measurements between the first- and second-wave DF.

In the present study, to mimic the second wave of follicular development which develops during a period when there are greater concentrations of P4, a new wave of follicular development was induced by stimulating ovulation from the DF in the first wave with gonadotropin releasing hormone (GnRH), and this wave was defined it as the second-wave model. Using this protocol, a similar time axis of duration of follicular growth and timing of maturation existed between the first- and second-wave of follicle development, and the maturation and changes occurring before ovulation were examined between the first- and second-wave DF.

The hypothesis was that the quality of DF is greater in the first- as compared with the second-wave of follicular development if the same time axis of duration of follicular growth and timing of maturation exists.

In this study, the objectives were to evaluate the dynamics of follicular growth, BF of the wall of the pre-ovulatory follicle, follicular fluid (FF) steroid hormones and gene expression of granulosa cells (GC) in the first- and second-wave DF in cows where there is a well-controlled time axis.

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals

Eight non-lactating Holstein cows (age, mean ± SEM, 5 ± 1.8 years; range 3–8 years) were used. The cows were kept under the normal management program of the Field Science Center of Obihiro University and were fed daily with a corn silage, hay, and concentrate diet, with free access to water. The cows underwent regular estrous cycles, and were clinically healthy. The experimental procedures complied with the *Guide for Care and Use of Agricultural Animals of Obihiro University*.

2.1.2. Study design

To examine growth and characteristics of DF in the first- and second-wave of follicular development, eight cows were divided into two groups (W1 and W2). In the W1 group, DF in the first follicular wave after spontaneous ovulation was examined. In the W2 group, DF in induced second follicular wave was examined. DF examined in W1 group and in W2 group were defined as DF-1 and DF-2, respectively. All eight cows were included in each groups in a cross over design (n = 8/group). Therefore, DF-1 and DF-2 were examined in different estrous cycle. Fig. 1 shows the timetable for the treatment of each experimental group. Spontaneous ovulation was confirmed in both groups using ultrasonographic scanning performed every 12 h. The DF-1 and the DF-2 were examined using ultrasonography.